Preliminary Application of Optical Trapping: Calculating Forces of Beads in Solution

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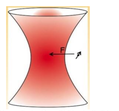
Abstract

Cell edge movements contribute to the tensions involved in developments of holes within amnioserosa cells of *Drosophila melanogaster* (fruit fly) embryos after heat shock treatments. However, characterizations of the mechanical tendencies of the cells prove to be problematic and the proposed solution is to measure the tension of cell edges using optical trapping. Optical trapping uses a tightly focused laser beam to generate forces capable of moving dielectric objects of microscopic size. The goal of this research is to execute the necessary preparations needed to obtain a strong and stable trap. During the research, a 445nm fiber-laser diode intended for trapping purposes was coupled into the optical pathway of an existing ablation laser. To enhance the accuracy of the laser alignment, the divergence of the 445nm laser was calculated. Simultaneously, protocols to focus the beam were followed. Sample slides of polystyrene beads and phosphate-buffered saline (PBS) solution were made to test trapping. Evidence of optical trapping were observed, however, the strength of the trap was inadequate for its application purpose. The next phase is to evaluate and enhance all aspects to generate a more tightly focused beam, which should lead to a better trap. Once trapping is achieve, the force will be determine using an ImageJ plugin, JavaScript code, to obtain the speed of cell-edge motion.

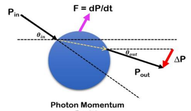
Background

❖ **Optical Trapping Background**

- Light carries momentum and energy.
- Optical trap is the exploitation of the momentum of photons to generate a force used to trap a dielectric particle[1].
- Ashkin was the first to show that a highly focused continuous laser can be use to trap micron-sized particles[2].
- The physical principles of optical trapping can be explained in two different regime[3].
  - ➢ Wavelength of light is greater than the size of trapping particle ($\lambda \gg d$)
  - ➢ Wavelength of light is less than the size of the trapping particle ($\lambda \ll d$)



Dipole Moment (Retrieved from: Bisd.education YouTube Channel, "Microscopy- Optical Traps: An Introduction by Carlos Bustamante")

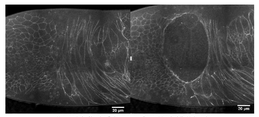


Snell's Law
$P_{in} \sin\theta_{in} = P_{out} \sin\theta_{out}$

1. Ashkin, A. (1970). Acceleration and Trapping of Particles by Radiation Pressure. Physical Review Letters, 24(4), 156-159.
2. Ashkin, A., Dziedzic, J., Bjorkholm, J., & Chu, S. (1986). Observation of a single-beam gradient force optical trap for dielectric particles. Optical Angular Momentum, 11(5), 288-290.
3. Nave, R. (n.d.). Dipole Moment. Retrieved July 26, 2015.

Objective

The Hutson Lab at Vanderbilt University performed heat shock experiments on *Drosophila Melanogaster* (fruit fly) embryos that led to the discovery of holes in the amnioserosa, which was caused by structural failure. The goal of this research is to obtain a strong and stable optical trap that can be used on the cell edges to observe and analyze cell tension and forces between cell-cell interactions.



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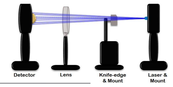
Methods

Sample Slides Preparation of Beads in PBS

▪0.5 and 0.05 microns size polystyrene bead solution was mixed separately into phosphate-buffered saline (PBS) in dilution ratios of 1:99 and 1:19 to make two 1µm sample slides.
▪Both sample slides are used for visual confirmation of optical trap.

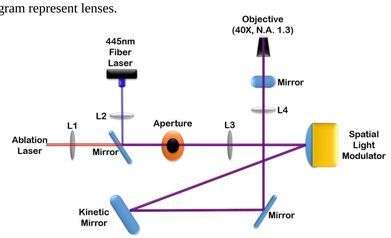
Knife-edge Measurement

- The 445nm fiber laser was shined onto a detector a distance of 11.2in and then 7.1in away.
- A sharp edge (blade) was moved across the laser beam in the step size of 0.001in.
- At each step size the intensity of the beam was recorded and used in divergence calculation



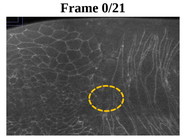
Optical Pathway Setup

▪The 445nm fiber laser was coupled to an existing beam path for an ablation laser.
▪Both beams were expanded, through individual beam expander set-up, and were fitted on the surface of the spatial light modulator (SLM) set-up.
▪A kinetic mirror and a positioning lens was used to guide the beam to the back of a confocal microscope's objective lens with a numerical aperture of 1.3.
▪L1, L2, L3, and L4 in diagram represent lenses.



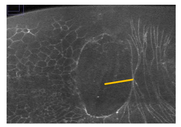
ImageJ Kymograph Plugin

Frame 0/21



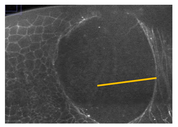
1. Pick relative area to start edge tracing

Frame 13/21

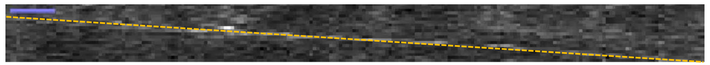


2. Trace cell motion by extending a line along its path.

Frame 21/21



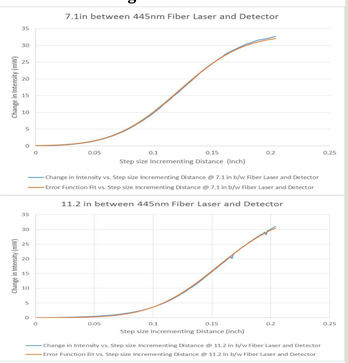
3. Complete the path trace at the last frame.



4. Run a Kymograph Plugin to produce a pixel-to-frame image of the cell-edge path. From here, obtaining the speed at which the cell-edge is moving is a matter of programing using JavaScript.

Results

Divergence Calculation



- $\left(\frac{P_1}{2}\right) * \left(1 + ERF\left(\sqrt{2}\right) * \frac{(X - P_2)}{P_3}\right)$, error function, and the plotted graph from the knife-edge measurement was used to find the fiber laser beam radius.
- The formula $\boldsymbol{\theta = 2\tan^{-1}\left(\frac{D_f - D_i}{2l}\right)}$ was used to find the diverging angle of the 445nm fiber laser beam.

Manufacturer Divergence: <1.1mrads
Calculated Divergence: 0.585mrad

Optical Trap Calculations

- Evidence of potential optical trap using the 445nm fiber laser was observed.
- The faintness of the cluster of beads led to the conclusion that the trap was very weak.
- Modification to optical set up needed.

Cell Edge Speed Programming

Program picks the highest most intense pixel in every frame to plot a pixel (distance) vs. frame (time) graph. The high intensity pixels in Kymograph image represent location of the cell edges at a specific frame. The best way to locate cell edges from the image is to incorporate a "running average of intensity". Speed is obtain from the graph.

Conclusions

To obtain a stable optical trap, the divergence of the 445nm fiber laser was calculated. The laser was coupled to the beam path of an existing ablation laser, collimated, and aligned to fill the back of a confocal microscope's objective. Sample slides of bead solution in PBS were use to visually confirm optical trap. There were evidence of trap, however, the dullness of the beads led to the conclusion that the trap was weak. Therefore, the optical setup must be modified to achieve a well focused beam that will increase the strength of the optical trap. Programming was only successful in finding high intensity pixels and not the running average from Kymograph image. The goal to determine the speed of cell-edge motion was not achieve.

Acknowledgements

Hutson Lab, Vanderbilt University; NIH Grant # 1R01GM099107 and 1R21AR068933; MARC/NIH/NIGMS Grant # 1T34GM100831 , NIH Grant #5T34GM100831; NSF Award #1358862; Vanderbilt University Physics and Astronomy Research Experience for Undergraduates (REU) Program 2015.